ACCELERATED PUBLICATION

Cbl–ArgBP2 complex mediates ubiquitination and degradation of c-Abl

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The mechanisms leading to the ubiquitination and degradation of the activated c-Abl kinase have not yet been identified. We found that the multi-adaptor protein ArgBP2 links c-Abl to the ubiquitin ligase Cbl. Phosphorylation of Cbl and ArgBP2 by c-Abl resulted in the stabilization of their interactions, thus facilitating Cbl-induced ubiquitination and subsequent degradation of c-Abl and ArgBP2.

Key words: Abl, ArgBP2, Cbl, degradation, tyrosine kinase, ubiquitination.

INTRODUCTION

c-Abl is a non-receptor tyrosine kinase involved in regulating cell growth, differentiation, re-organization of the actin cytoskeleton and apoptosis [1]. c-Abl and c-Arg form the Abelson family of protein tyrosine kinases [2]. The activity of c-Abl is tightly regulated in the cell by numerous signals [3], and deregulation of its activity, as found in the BCR/Abl fusion protein [4], leads to the development of cancer. Recent findings showed that the terminal inactivation of c-Abl is mediated by ubiquitin-dependent degradation in the proteasome [5]. However, the molecular mechanisms underlying this process are unknown. Cbl acts as a ubiquitin ligase and contains several functional domains that are able to interact with a number of signalling proteins [6], including c-Abl [7]. Cbl was shown to negatively regulate signalling by receptor and non-receptor type tyrosine kinases [8]. Indeed, recent reports showed that Cbl controls down-regulation of activated receptor tyrosine kinases by mediating receptor ubiquitination [6], as well as by recruiting adaptor protein CIN85 and endophilins in the complex with activated receptors [9–11]. This last finding shows that associated adaptor proteins may contribute to the function of Cbl. Using a two-hybrid screen with Cbl as bait, we found that the Arg-binding protein 2 (ArgBP2) [12] is a new Cbl-interacting protein. ArgBP2 is an adaptor protein containing a SoHo (Sorbin Homology) domain, which is implicated in binding to the lipid raft protein flotillin [13], and three Src homology (SH) 3 domains, which are known to interact with polyproline motifs of c-Arg and c-Abl [12]. Interestingly, ArgBP2 is also a substrate for both c-Abl and c-Arg kinases [12]. In the present report, we show that ArgBP2 negatively regulates Abl kinases by recruiting Cbl in the complex with c-Abl, facilitating phosphorylation of Cbl by c-Abl and promoting Cbl-directed ubiquitination and degradation of c-Abl.

EXPERIMENTAL

Cells and reagents

ArgBP2-A cDNA and antibody were provided by G. Kruh (Medical Science Division, Fox Chase Cancer Center, Philadelphia, PA, U.S.A.). ArgBP2-CT antibody was generated using the three SH3 domains of ArgBP2 fused to glutathione S-transferase (GST) as an antigen. FLAG-tagged ubiquitin in pcDNA3.1 and Cbl-constructs have been described previously [9]. The expression vectors coding for Abl, kinase-deficient Abl (Abl-KD) and an Abl SH2 domain mutant (Abl-SH2M) were provided by A. M. Pendergast (Department of Pharmacology, Duke University, Durham, NC, U.S.A.) and G. Superti-Furga (EMBL, Heidelberg, Germany). pRK5-Nck cDNA was provided by J. Schlessinger (Department of Pharmacology, Yale University, New Haven, CT, U.S.A.). HEK-293T cells have been described previously [9], and CHO-K1 and K562 cells were purchased from American Type Culture Collection (Manassas, VA, U.S.A.). CHO cells stably expressing myc-ArgBP2 were isolated following the transfection of pcDNA2-ArgBP2-Myc in parental CHO cells and subsequent selection in medium containing 2.4 mg/ml G418.

Two-hybrid screen

The yeast screening was performed using the GAL4-based Matchmaker two-hybrid system with the human foetal brain, thymus and T cell libraries (Clontech). pYTH9 GAL4-DNA binding domain vector with human Cbl was used as bait. The yeast re-transformation and liquid β-galactosidase assay were performed as described in the Matchmaker system manual.

Transfection

293T and CHO cells were transfected using Lipofectamine reagent (Invitrogen) following the manufacturer's instructions. A total of 6 mg of cDNA was used when cells were transfected in 10 cm dishes, and 1 mg per well when transfected in 6-well plates. Cells were lysed in lysis buffer (24–48 h post-transfection) and centrifuged for 15 min at 4 °C. Cleared lysates were used for immunoprecipitation, GST pull-down assays and Western blotting analyses.

Immunoprecipitation, immunoblotting and GST pull-down assays

The following antibodies were used: rabbit polyclonal anti-ArgBP2 [raised against the C-terminal (CT) of ArgBP2], mouse monoclonal anti-phosphotyrosine (anti-PY; PY99, Santa Cruz Biotechnology), anti-haemagglutinin (anti-HA; 12CA5, Roche), anti-myc (9E10, Santa Cruz Biotechnology) and anti-FLAG (M2 and M5, Sigma) antibodies, goat polyclonal anti-GST (Pharmacia), rabbit polyclonal anti-Abl (K12, Santa Cruz

Abbreviations used: Abl-KD, kinase-deficient Abl; Abl-SH2M, Abl SH2 domain mutant; ArgBP2, Arg-binding protein 2; Cbl-70Z, ubiquitin-ligase-deficient mutant of Cbl; CT, C-terminal; GST, glutathione S-transferase; HA, haemagglutinin; PY, phosphotyrosine; RF, ring finger; SH, Src homology; SoHo, sorbin homology.

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Biotechnology) and anti-Cbl {raised against the ring finger (RF) domain of Cbl [9]}. Lysates with adjusted protein concentrations (Bradford assay, BioRad) were incubated with antibody for 2 h at 4 °C. Immune complexes were precipitated following a 1 h incubation with Protein A–Sepharose beads. After washing in cold lysis buffer, the complexes were resuspended in Laemmli sample buffer (BioRad), boiled and resolved by SDS/PAGE. Proteins were transferred on to nitrocellulose filters and blotted in Tris-buffered saline/5% BSA containing anti-Cbl, anti-PY, anti-myc, anti-HA and anti-GST antibodies at 1:500, anti-Abl antibody at 1:250, anti-ArgBP2 antibody at 1:1000 and anti-(ArgBP2-CT) antibody at 1:250. For the GST-binding assays, GST-fusion proteins adsorbed on glutathione–Sepharose beads were added to cell lysates, incubated with the lysates for 2 h at 4 °C, washed in the lysis buffer, and were processed for Western blotting as described above. To determine the turnover of Abl, CHO cells were transfected with either c-Abl and Cbl, or with c-Abl and Cbl plus ArgBP2. Following transfection (48 h later), cells were treated with 10 μg/ml cycloheximide in serum-free medium for varying times. c-Abl blots were exposed to a CCD camera (Fujifilm LAS-1000 Plus) and signal quantification was performed using AIDA software. Values were expressed as a percentage of the initial signal and Figure 4(C) shows both values and trend lines for both series of values.

RESULTS AND DISCUSSION

In order to identify new binding partners for Cbl, we performed a yeast two-hybrid screening assay with Cbl as bait. Among several double-positive clones (positive for the ability to grow under selective conditions and for β-galactosidase activity), we focused on one clone that encoded the adaptor protein ArgBP2, which was previously shown to bind to the Abelson kinases, Abl and Arg [12]. The SH3 domains of ArgBP2 potently interacted with the C-, but not the N-, terminal half of Cbl in yeast cells, as measured by a liquid β-galactosidase assay (Figure 1A). To further characterize the interactions between Cbl and ArgBP2, we created GST-fusion proteins encoding individual or combinations of the three SH3 domains of ArgBP2. Individual SH3B and SH3C domains, but not SH3A, bound as efficiently as all three SH3 domains of ArgBP2 (SH3ABC) to Cbl (Figure 1B). The SH3B domain interacted with Cbl-563 (the first 563 amino acids of Cbl) but not with Cbl-528, whereas SH3C was able to bind Cbl-528, but not Cbl-480 (Figure 1C). These results suggest that SH3B and SH3C of ArgBP2 may bind to distinct parts of the proline rich region of Cbl (Figure 1D). On the other hand, the SH3A and SH3C domains of ArgBP2 were shown previously to bind to polyproline motifs in the C-terminus of Abl and Arg [12], indicating that distinct SH3 domains of ArgBP2 bind to both Cbl and Abl.

These results led us to hypothesize that endogenous ArgBP2 may act as a linker between Cbl and c-Abl in mammalian cells. We tested this hypothesis in the K562 cell line derived from a patient with chronic myelogenous leukaemia [14]. These cells harbour the chromosomal translocation that results in a high expression of a constitutively active BCR–Abl fusion protein. Cbl associates with, and is a major substrate of, BCR–Abl [15]. Indeed, Cbl was found in complex with both BCR–Abl and, to a lesser extent, with c-Abl (Figure 2A). Importantly, Cbl and ArgBP2 were also able to co-precipitate each other in lysates of

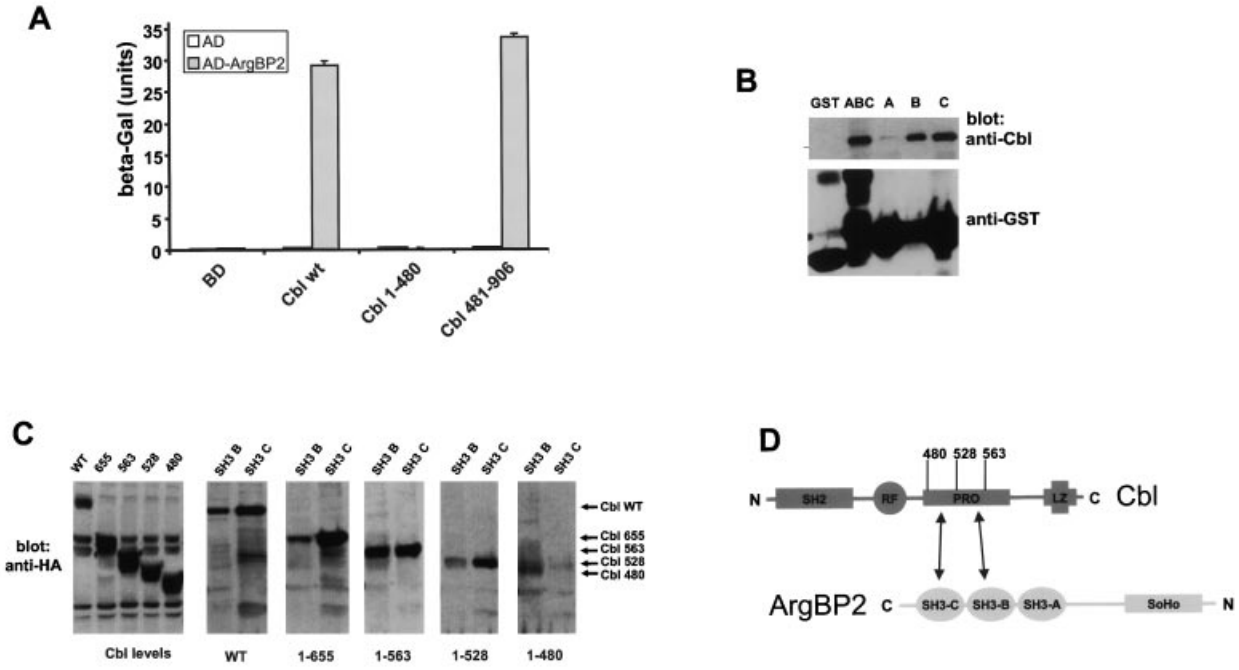

Figure 1 ArgBP2 is a new Cbl-interacting protein

(**A**) Yeast re-transformation and β-galactosidase assay. ArgBP2 in the pACT2 vector was introduced into the Y190 yeast strain, expressing either the empty pYTH9 vector or pYTH9 containing indicated Cbl constructs, and the β-galactosidase assay was performed as described in the Experimental section. AD, GAL-4 activation domain; BD, GAL-4 DNA-binding domain; wt, wild-type. (**B**) GST pull-down of Cbl using SH3ABC (all three domains) or individual SH3A, B or C domains of ArgBP2 fused to GST. Filters were blotted with anti-Cbl and anti-GST antibodies. (**C**) GST pull-down assay of HA-tagged C-terminal deletion forms of Cbl using GST-SH3B or GST-SH3C domains of ArgBP2. Filters were blotted using an anti-HA antibody. WT, wild-type. (**D**) Schematic representation of a model of interaction between ArgBP2 and Cbl. PRO, proline rich region; LZ, leucine zipper.

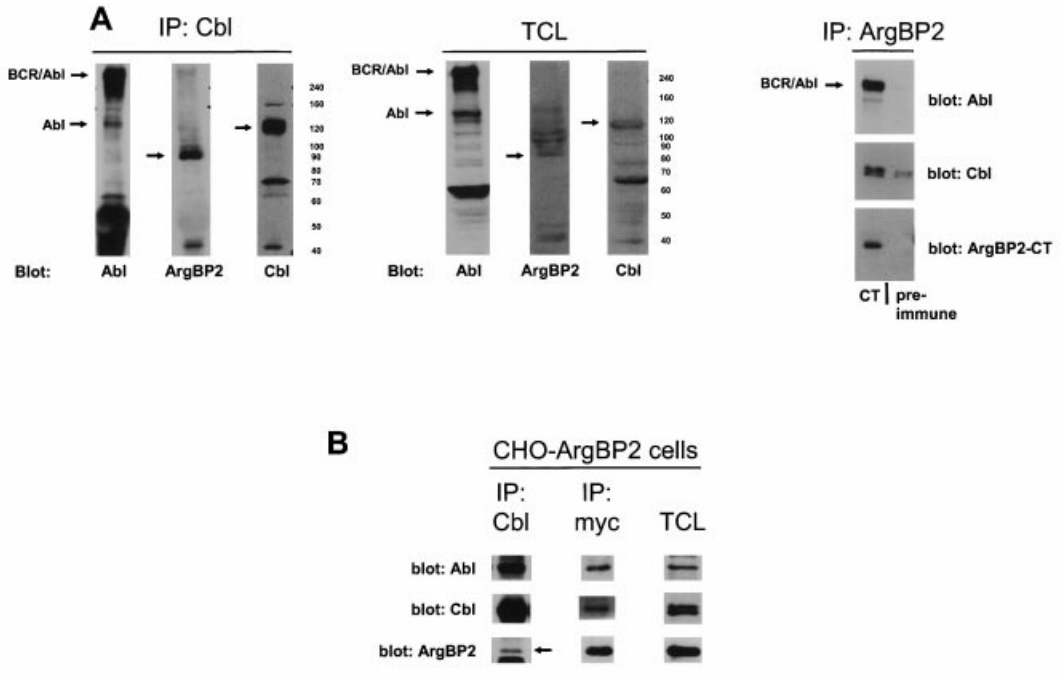

Figure 2 Formation of Cbl–ArgBP2–Abl complexes in mammalian cells

(**A**) Total cell lysates from K562 cells were incubated with anti-Cbl or anti-(ArgBP2-CT) antibodies and precipitated proteins were resolved by SDS/PAGE, transferred to nitrocellulose membranes and subjected to immunoblotting with anti-ArgBP2, anti-Abl or anti-Cbl antibodies. (**B**) Lysates of CHO-Myc-ArgBP2 cells were subjected to immunoprecipitation with anti-Myc (Myc-ArgBP2) or anti-Cbl antibodies followed by immunoblotting with indicated antibodies. IP, immunoprecipitation; TCL, total cell lysates.

these cells (Figure 2A), suggesting that Cbl can form complexes with BCR–Abl, c-Abl and ArgBP2 in transformed leukaemia cells. Moreover, we were also able to detect the formation of a Cbl–ArgBP2–cAbl complex in non-transformed cell lines, such as CHO cells stably expressing myc-ArgBP2 (Figure 2B). Taken together, our results identified ArgBP2 as a new Cbl-interacting protein, able to form complexes with both Cbl and c-Abl in mammalian cells.

In order to determine whether the complex between Cbl and c-Abl is critically dependent on the presence of ArgBP2, we co-transfected Cbl, c-Abl and increasing amounts of ArgBP2, and analysed the ability of Cbl to co-precipitate c-Abl. As shown in Figure 3(A), enhanced co-precipitation between Cbl and c-Abl correlated with the increasing amounts of ArgBP2 expressed in HEK293T cells, while Nck, another adaptor protein containing three SH3 domains that was shown to bind to both Cbl and c-Abl [16], led to inhibition in their co-precipitation (Figure 3A). In addition, Cbl and ArgBP2 were also tyrosine-phosphorylated by c-Abl in these complexes (Figures 3B and 3C). Co-expression of ArgBP2 increased c-Abl-induced phosphorylation of transfected and endogenous Cbl (Figures 3B and 3D). This was most probably due to the ArgBP2-dependent recruitment of Cbl with c-Abl (Figure 3A). Likewise, the binding of ArgBP2 to wild-type c-Abl, which induces tyrosine phosphorylation of ArgBP2 (Figure 3C), was significantly more potent than the interaction between ArgBP2 and Abl-KD (Figure 3C). These results indicate that, in addition to constitutive binding of the SH3 domains of ArgBP2 to proline-rich sequences of c-Abl [12], further interactions, probably involving the binding of the SH2 domain of c-Abl to phosphorylated tyrosines of ArgBP2, may occur. To test this hypothesis, we compared the ability of wild-type c-Abl and Abl-SH2M to bind to and phosphorylate ArgBP2 and Cbl. Mutation of the SH2 domain of c-Abl led to a decreased formation of a Cbl–ArgBP2–Abl complex when compared with cells expressing wild-type c-Abl (Figure 3D). The impairment in complex formation was also accompanied by a decrease in c-Abl-induced phosphorylation of Cbl and ArgBP2 (Figure 3D). This last finding mimics the inhibitory effect of Nck on Cbl–c-Abl interactions, which has been shown to result from a competition of SH2 domains of Nck and c-Abl for binding to Cbl [16]. Taken together, these results suggest the existence of a positive loop of phosphorylation-dependent interactions between Cbl, ArgBP2 and c-Abl that finally leads to stabilization of these interactions, and possibly the creation of a trimeric complex.

It has been reported recently that activated c-Abl is degraded via a ubiquitin/proteasome-dependent pathway [5]. Since Cbl was shown to mediate ubiquitination of a number of activated receptor and non-receptor protein tyrosine kinases [17–19], we tested whether Cbl, when associated with ArgBP2 and c-Abl, could also promote ubiquitination and degradation of c-Abl. Cbl-directed ubiquitination of c-Abl was observed in cells expressing Cbl and c-Abl, and was significantly enhanced when ArgBP2, but not Nck, was co-expressed (Figure 4A). Interestingly, ArgBP2 was also ubiquitinated by Cbl (Figure 4A), suggesting that Cbl-induced ubiquitination of c-Abl, as well as ArgBP2, is dependent on their mutual interactions.

Ubiquitination of proteins is known to target them for degradation by the 26S proteasome and/or lysosome [8]. Therefore we next investigated whether Cbl-mediated ubiquitination of c-Abl was followed by its degradation, and whether the presence of ArgBP2 promoted degradation of c-Abl. We could not detect significant degradation of ubiquitinated c-Abl in HEK293T cells (Figure 4A), possibly due to very high over-expression of transfected proteins in these cells. We therefore

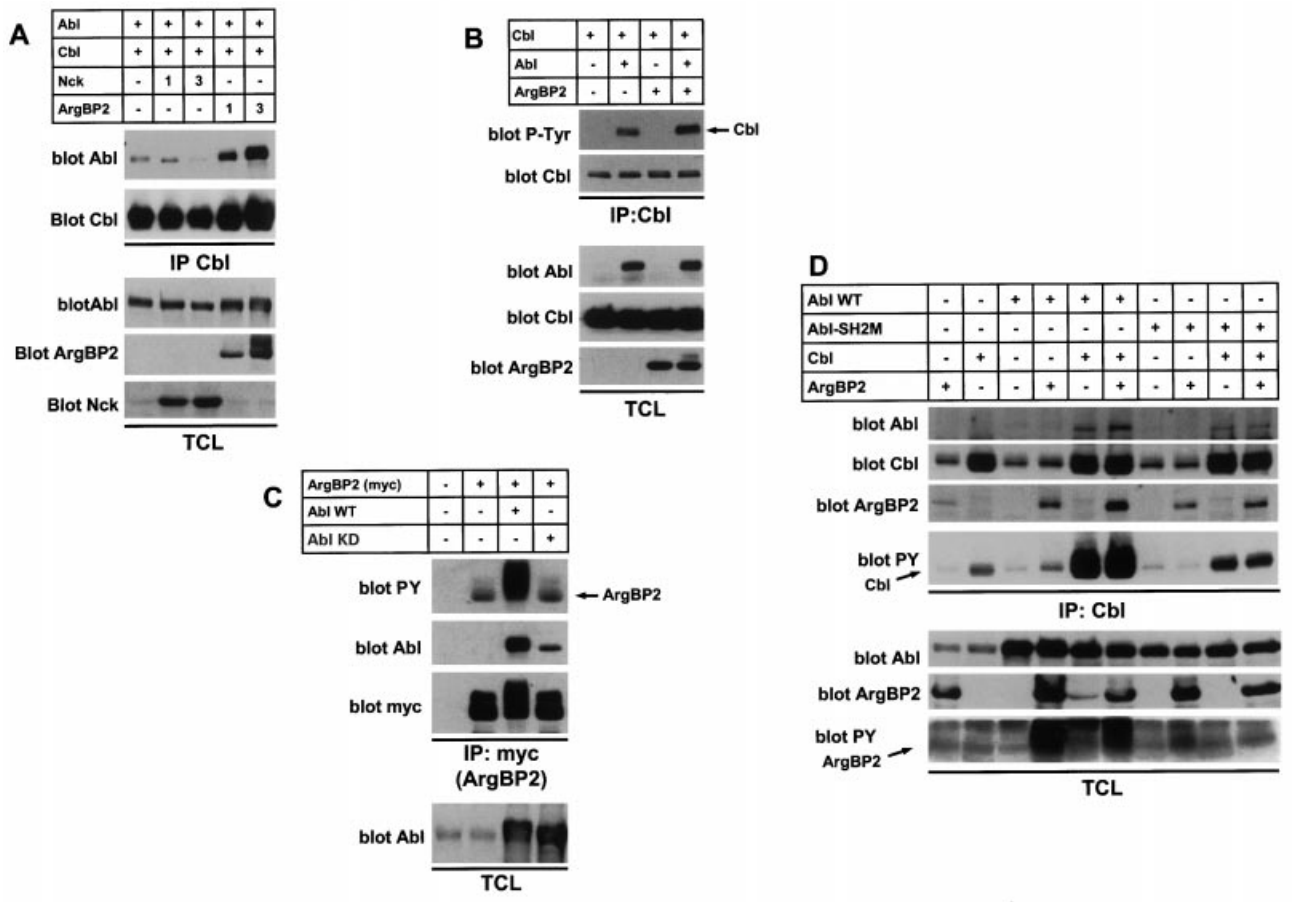

Figure 3 ArgBP2 links c-Abl and Cbl

(**A**) ArgBP2, but not Nck, facilitates the interactions between c-Abl and Cbl. HEK293T cells were transfected with plasmids coding for Cbl, c-Abl, and increasing concentrations of ArgBP2 or Nck. Total cell lysates or Cbl immunoprecipitates were blotted with indicated antibodies. (**B**) ArgBP2 facilitates phosphorylation of Cbl by c-Abl. Lysates of HEK293T cells expressing Cbl in combination with c-Abl and/or ArgBP2 (total cell lysates) or Cbl immunoprecipitates were probed with anti-phosphotyrosine, anti-Abl, anti-ArgBP2 or anti-Cbl antibodies. (**C**) Interactions between c-Abl and ArgBP2 are increased by the kinase activity of c-Abl. HEK923T cells were transfected with ArgBP2, Abl-WT, and Abl-KD as indicated. Lysates were blotted with anti-Abl antibodies or subjected to immunoprecipitation with anti-myc antibody (ArgBP2), followed by immunoblotting with indicated antibodies. (**D**) The SH2 domain of c-Abl is critical for stabilization of the Cbl-ArgBP2-Abl interactions. HEK293T cells were transfected with either wild-type c-Abl (Abl WT) or c-Abl containing a point mutation in the SH2 domain (Abl-SH2M), in combination with ArgBP2 and/or Cbl. Cell lysates were subjected to immunoprecipitation with anti-Cbl antibodies and filters were blotted with anti-PY, anti-Abl, anti-Cbl and anti-ArgBP2 antibodies. IP, immunoprecipitates; TCL, total cell lysates.

studied degradation of c-Abl in CHO cells in which transient transfection leads to more physiological levels of expressed proteins. A decrease in the protein level of the c-Abl kinase was observed when c-Abl was co-expressed with either ArgBP2 or Cbl (Figure 4B), which parallels their effect on the ubiquitination of c-Abl (Figure 4A). Co-expression of both Cbl and ArgBP2 together with c-Abl led to almost complete destruction of c-Abl (Figure 4B). Furthermore, expression of Cbl together with ArgBP2 resulted in a significantly shorter half-life of c-Abl, when compared with the half-life of c-Abl in cells expressing only Cbl and c-Abl (Figure 4C). On the other hand, expression of Cbl-70Z, the ubiquitin-ligase-deficient mutant form of Cbl [15], was unable to affect the stability of c-Abl. In addition, the protein level of ArgBP2 was also strongly reduced in the presence of Cbl, but not Cbl-70Z, and it was further decreased when c-Abl was co-expressed in the cells (Figure 4B), suggesting that ArgBP2 and c-Abl may be targeted for destruction via a common pathway. Furthermore, Cbl-induced degradation of Abl and ArgBP2 is dependent on the proteasome pathway, since inhibitors of proteasome block degradation of both Abl and ArgBP2 (results not shown). This is also consistent with findings that other c-Abl-interacting proteins, such as Abi-1/2, are also degraded by Abl kinases in a proteasome-dependent manner [20].

In conclusion, we have demonstrated that ArgBP2 acts as a critical regulator of mutual interactions between c-Abl and Cbl in mammalian cells, leading to Cbl-directed ubiquitination and degradation of active c-Abl kinase. Understanding the spatial and temporal constraints under which these interactions take place following various external stimuli is one of the challenges for future investigations. It was shown that c-Abl generates a panel of different, sometimes opposing, signals depending on its subcellular localization in the cell [1]. c-Abl appears to be involved in the modulation of the actin cytoskeleton via direct interactions with F-actin or other cytoskeletal proteins, including Mena and Wave [1,21]. These processes are highly dynamic and depend on localized cycles of stimulatory and inhibitory signals that modulate c-Abl activity. ArgBP2 and Cbl are also localized in the actin structures in the cell [6,12] and may thus provide a local signal to terminate activity of c-Abl. Our data also indicate that a co-ordinated regulation of two post-translational modi-

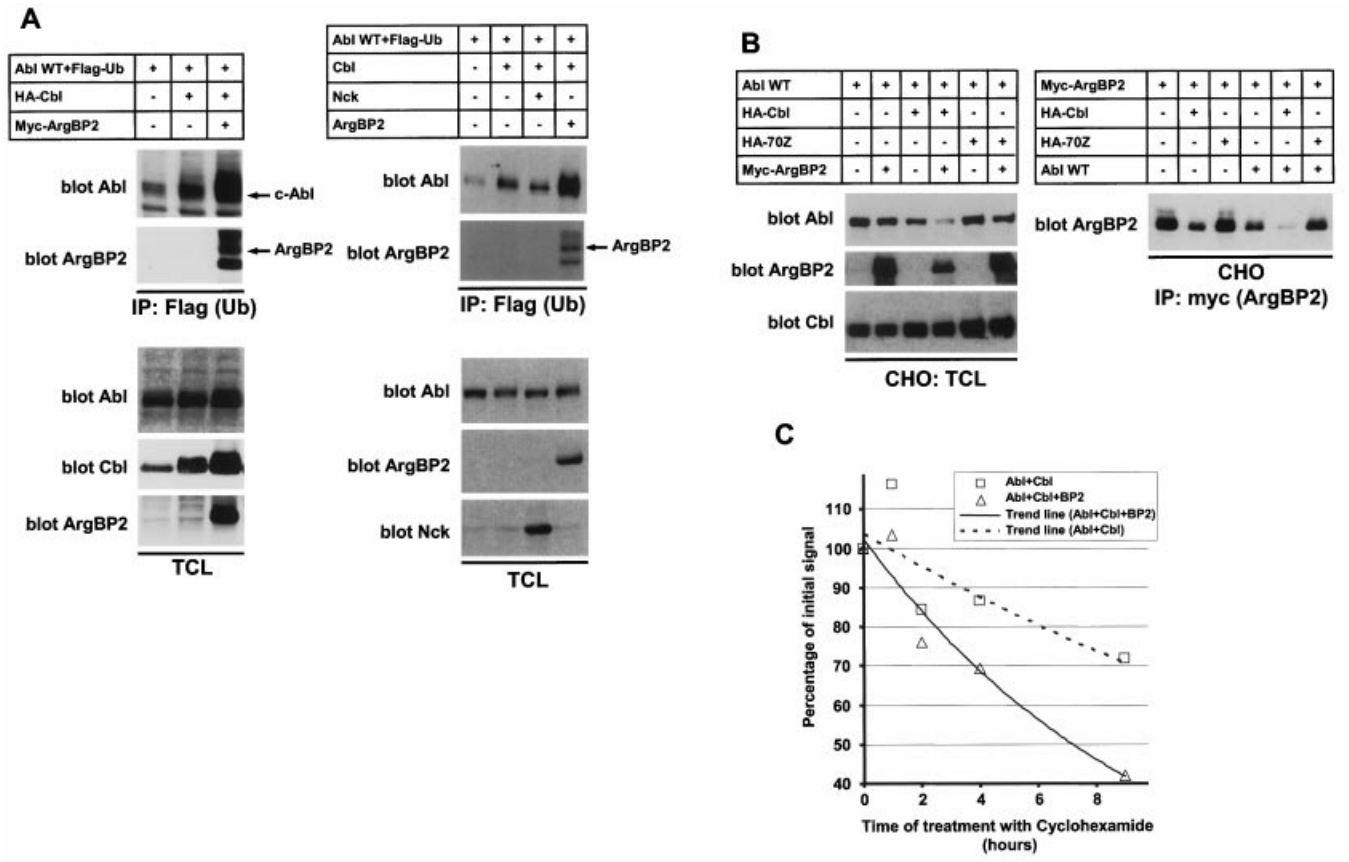

Figure 4 ArgBP2 enhances Cbl mediated ubiquitination and degration of c-Abl

(**A**) HEK293T cells were transfected with FLAG-tagged ubiquitin (Ub) and c-Abl in combination with ArgBP2, Cbl or Cbl-70Z. Lysates were subjected to immunoprecipitation (IP) using anti-FLAG antibodies, and filters were blotted with anti-Abl and anti-ArgBP2 antibodies. Total cell lysates (TCL) were blotted with anti-Abl, anti-ArgBP2 and anti-Cbl antibodies. (**B**) In order to monitor the stability of the transfected proteins, CHO cells were transfected with combinations of c-Abl (Abl WT), Cbl (HA-Cbl), Cbl-70Z (HA 70Z) and ArgBP2 (Myc-ArgBP2) as indicated. Total cell lysates were then blotted for c-Abl, ArgBP2 and Cbl, and anti-myc immunoprecipitates were blotted with anti-ArgBP2 antibodies. (**C**) To study the effect of ArgBP2 on Cbl-induced degradation rate of c-Abl, CHO cells were transfected with plasmids coding for Cbl, ArgBP2 and c-Abl, or only Cbl and c-Abl. Cells were treated with protein synthesis inhibitor cycloheximide and the protein levels of c-Abl were monitored for the indicated time points. Results were quantified and are shown as the percentage of Abl level that remains in the cells following inhibition of protein synthesis.

fications, such as phosphorylation and ubiquitination, are necessary for terminal inactivation of c-Abl. Thus tight regulation of these mechanisms may be essential to prevent aberrant activation of protein tyrosine kinases and subsequent cell transformation.

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REFERENCES

1 Van Etten, R. A. (1999) Cycling, stressed-out and nervous: cellular functions of c-Abl. Trends. Cell. Biol. **9**, 179–186
2 Kruh, G. D., Perego, R., Miki, T. and Aaronson, S. A. (1990) The complete coding sequence of arg defines the Abelson subfamily of cytoplasmic tyrosine kinases. Proc. Natl. Acad. Sci. U.S.A. **87**, 5802–5806
3 Pluk, H., Dorey, K. and Superti-Furga, G. (2002) Autoinhibition of c-Abl. Cell (Cambridge, Mass.) **108**, 247–259
4 Gotoh, A. and Broxmeyer, H. E. (1997) The function of BCR/ABL and related proto-oncogenes. Curr. Opin. Hematol. **4**, 3–11
5 Echarri, A. and Pendergast, A. M. (2001) Activated c-Abl is degraded by the ubiquitin-dependent proteasome pathway. Curr. Biol. **11**, 1759–1765
6 Thien, C. B. and Langdon, W. Y. (2001) Cbl: many adaptations to regulate protein tyrosine kinases. Nat. Rev. Mol. Cell. Biol. **2**, 294–307
7 Shishido, T., Akagi, T., Ouchi, T., Georgescu, M. M., Langdon, W. Y. and Hanafusa, H. (2000) The kinase-deficient Src acts as a suppressor of the Abl kinase for Cbl phosphorylation. Proc. Natl. Acad. Sci. U.S.A. **97**, 6439–6444
8 Bonifacino, J. S. and Weissman, A. M. (1998) Ubiquitin and the control of protein fate in the secretory and endocytic pathways. Annu. Rev. Cell. Dev. Biol. **14**, 19–57
9 Soubeyran, P., Kowanetz, K., Szymkiewicz, I., Langdon, W. Y. and Dikic, I. (2002) Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. Nature (London) **416**, 183–187
10 Petrelli, A., Gilestro, G. F., Lanzardo, S., Comoglio, P. M., Migone, N. and Giordano, S. (2002) The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. Nature (London) **416**, 187–190
11 Dikic, I. (2002) CIN85/CMS family of adaptor molecules. FEBS Lett. **529**, 110–115
12 Wang, B., Golemis, E. A. and Kruh, G. D. (1997) ArgBP2, a multiple Src homology 3 domain-containing, Arg/Abl-interacting protein, is phosphorylated in v-Abl-transformed cells and localized in stress fibers and cardiocyte Z-disks. J. Biol. Chem. **272**, 17542–17550
13 Kimura, A., Baumann, C. A., Chiang, S. H. and Saltiel, A. R. (2001) The sorbin homology domain: a motif for the targeting of proteins to lipid rafts. Proc. Natl. Acad. Sci. U.S.A. **98**, 9098–9103
14 Ben-Neriah, Y., Daley, G. Q., Mes-Masson, A. M., Witte, O. N. and Baltimore, D. (1986) The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. Science (Washington, D.C.) **233**, 212–214
15 Andoniou, C. E., Thien, C. B. and Langdon, W. Y. (1994) Tumour induction by activated abl involves tyrosine phosphorylation of the product of the cbl oncogene. EMBO J. **13**, 4515–4523

16 Miyoshi-Akiyama, T., Aleman, L. M., Smith, J. M., Adler, C. E. and Mayer, B. J. (2001) Regulation of Cbl phosphorylation by the Abl tyrosine kinase and the Nck SH2/SH3 adaptor. Oncogene **20**, 4058–4069
17 Andoniou, C. E., Lill, N. L., Thien, C. B., Lupher, Jr, M. L., Ota, S., Bowtell, D. D., Scaife, R. M., Langdon, W. Y. and Band, H. (2000) The Cbl proto-oncogene product negatively regulates the Src-family tyrosine kinase Fyn by enhancing its degradation. Mol. Cell. Biol. **20**, 851–867
18 Rao, N., Ghosh, A. K., Ota, S., Zhou, P., Reddi, A. L., Hakezi, K., Druker, B. K., Wu, J. and Band, H. (2001) The non-receptor tyrosine kinase Syk is a target of Cbl-mediated ubiquitylation upon B-cell receptor stimulation. EMBO J. **20**, 7085–7095
19 Rao, N., Miyake, S., Reddi, A. L., Douillard, P., Ghosh, A. K., Dodge, I. L., Zhou, P., Fernandes, N. D. and Band, H. (2002) Negative regulation of Lck by Cbl ubiquitin ligase. Proc. Natl. Acad. Sci. U.S.A. **99**, 3794–3799
20 Dai, Z., Quackenbush, R. C., Courtney, K. D., Grove, M., Cortez, D., Reuther, G. W. and Pendergast, A. M. (1998) Oncogenic Abl and Src tyrosine kinases elicit the ubiquitin-dependent degradation of target proteins through a Ras-independent pathway. Genes Dev. **12**, 1415–1424
21 Westphal, R. S., Soderling, S. H., Alto, N. M., Langeberg, L. K. and Scott, J. D. (2000) Scar/WAVE-1, a Wiskott-Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. EMBO J. **19**, 4589–4600

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